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Antipediculicidal activity of seed extracts of *Annona muricata* Linn.

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Abstract

A study was carried out to investigate the phytochemical constituents, *in vitro* and *in vivo* antipediculicidal activity of *Annona muricata* Linn. seeds against lice infestation in backyard poultry. The dried powdered seeds of *A. muricata* Linn. were extracted, using petroleum ether, ethyl acetate and methanol by a Soxhlet extractor and preliminary phytochemical screening was performed, using standard protocols. *In vitro* antipediculicidal activity of petroleum ether, ethyl acetate and methanolic seed extracts of various concentrations (12.5 mg/ml, 25.0 mg/ml, 50.0 mg/ml and 100.0 mg/ml), used and analyzed for average percentage mortality. Similarly, *in vivo* antipediculicidal activity was assessed in backyard poultry and premises. Preliminary screening revealed the presence of bioactive compounds, especially tannins, saponins, flavonoids, betacyanins, quinones, cardiac glycosides, terpenoids, phenols, coumarins, steroids and alkaloids in all extracts. Ethyl acetate extract showed more positivity for tannins, saponins, flavonoids, betacyanins, phenolics and alkaloids than petroleum ether extract. However, methanolic extract showed no strong positivity of bioactive compounds. *In vitro* antipediculicidal activity showed ethyl acetate had highest average mortality percentage rather than petroleum ether and methanolic extracts. Highest concentration of drugs in all extracts performed better than lowest concentration. *In vivo* study of ethyl acetate (highest concentration), *i.e.,* 100.0 mg/ml had complete recovery of lice infestation in poultry and its premises. The results of the present study indicate that the seed extracts of *A. muricata* Linn. exhibited strong antipediculicidal activity and, thus, it is a good source of acaricidal activity.

Key words : *Annona muricata* Linn, antipediculicidal, backyard poultry, lice, phytochemical

1. Introduction

Ectoparasites are major threat for animals and human beings as they are external parasites, affect the health status of livestock and birds and act as vector for many diseases, zoonotic and economically significant. Ectoparasites include suborder ixodida (ticks), and orders siphonaptera (fleas) and phthiraptera (lice) (Bowman, 2009). Lice are small parasites which infest birds around the vent and the neck. Lice eggs bundle up to the size of a marble, becoming attached to the base of the feather (Brigid *et al.,* 2015). Poultry lice eat dead skin and feather dander and can cause severe irritation and stress, cause anemia, fever and carry some bacterial and viral diseases. Birds often stop laying. Lice infestation is much of clinical importance as they transmit and serve as reservoir of pathogens like swinepox and rickettsia (Wolf, 2010). Their chewing and blood feeding habit will result to anaemia, skin irritation and decrease in production capacity among poultry and livestock (Wall and Shearer, 1997). Therefore, ectoparasites are serious pest with vital role in disseminating vector-borne diseases, decreasing animal production and debilitating animal welfare (Fuehrer *et al.,* 2012; Campbell-Lendrum *et al.,* 2015).

Plants may be an alternative to the currently used acaricidal and insecticidal agents to control ticks, lice, mites and specific pests, because they constitute a rich source of bioactive chemicals (Isman, 2000) and to overcome drug resistance. The present study dealt with severe lice infestation in backyard desi birds and successive herbal treatment and efficacy of *A. muricata* Linn. (Annonaceae). *A. muricata* Linn. belongs to the family of Annonaceae, has a wide-spread pantropical distribution and has been pridely known as corossol. It is a widespread small tree and has its native in Central America (Alassane *et al.,* 2004). The fruit of *A. muricata* Linn. is found to be edible in Yunnan province of China (Chao-Ming *et al.,* 1998) and their fruits are used commercially for the production of juice, candy and sharbats. Powdered seeds and immature fruits of *A. muricata* Linn. can be administered topically to animals against fly larvae (myiases), lice and other insects. Annonaceae extracts have demonstrated for their entomological potential against lice infestations in backyard poultry. Intensive chemical investigations of the leaves and seeds of this species, have resulted in the isolation of a great number of acetogenins (Gajalakshmi *et al*., 2012). The isolated compounds display some of the interesting biological or the pharmacological activities, such as antitumoral, cytotoxicity, antiparasitic and pesticidal properties. Roots of these species are used in traditional medicine, due to their antiparasitical and pesti-

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cidal properties (Christophe *et al.,* 1997). Studies done on the leaves of *A. muricata* Linn. has been resulted in the isolation of eight cytotoxic acetogenins (Guem-soog *et al.,* 1998). The seed of this plant is well known for killing head lice in many countries (Boonyaprapasara *et al.,* 1998).

Therefore, much effort has been focused on plants or phytochemicals as a potential source of commercial acaricidal agents or as bioactive chemical compounds in this family, Annonaceae (Kim *et al.,* 2001; Lee *et al.,* 2001; Kim *et al.,* 2003). Considering this fact, present investigation has been designed to find out *in vitro* and *in vivo* acaricidal activity of *A. muricata* Linn. against lice infestation in backyard poultry.

2.1 Preparation of seed extract of *A. muricata* Linn.

The fully matured fresh seeds of the fruit *A. muricata* Linn. were collected from local market, Ramanathapuram, separated and were washed thoroughly with running tap water and shade dried for one weak and powered with the help of blender. The dried seed powder of *A. muricata* Linn. was extracted with methanol, petroleum ether and ethyl acetate separately. 100 ml of each solvent is mixed with 10 grams of seed powder and kept in mechanical shaker for 48 h. at room temperature. Extracts were then filtered by using Whatman filter paper No. 1. Extracts were concentrated in rotary evaporator at 40^{o}C and dried. All the extracts were stored in the refrigerator at 4°C until use. The extracted powder was dissolved in 10 % dimethyl sulfoxide (DMSO) for the further use.

2.2 Phytochemical screening of *A. muricata* Linn.

The phytochemical screening of seed extracts was assessed by standard phytochemical methods (Siddiqui and Ali, 1997; Savithramma *et al.,* 2011) at Botany Department, AVVM Sri Pusham College, Thanjavur, Tamil Nadu. Qualitative analysis of seed extracts was done, using petroleum ether, methanol and ethyl acetate to identify the major phytoconstituents such as tannins, saponins, flavonoids, anthocyanins, betacyanins, quinones, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, steroids and alkaloids.

2.3 Collection and processing of lice

The study was under taken from backyard desi poultry and household in and around Ramanathapuram District, Tamil Nadu. The desi birds with ectoparasitic infestation were examined for the presence of lice at various predilection sites and subjected for *in vitro* and *in vivo* clinical trials. The flock was selected at random, depending upon their availability and locality. Lice were collected from the predilection sites of the body by hand manipulation or with aid of blunt pointed forceps to avoid any harm to lice and host. These collected lice are put in vials and labeled with all details of flock owner, location of village, bird's details such as sex, age group and date of collection. The vials with lice were wrapped in cotton net cloth for oxygen supply and transported to Veterinary University Training and Research Centre (VUTRC), Ramanthapuram laboratory where they were placed in vials containing Alcohol-Formalin-Acetic acid (AFA) for preservation. Afterwards, they were processed and mounted in the slide following the technique (Edurado, 2012) and identified as (Figures 1 and 2).

2.4 *In vitro* antipediculicidal activity

In vitro antipediculicidal activity of various solvents petroleum ether, methanol and ethyl acetate seed extracts of *A. muricata* Linn. was evaluated on lice infestation, collected from desi birds. The collected lice were randomly divided several groups for the solvents petroleum ether, methanol and ethyl acetate seed extract were subjected for the study. The pour on method was used as described (Vihan and Agarwal, 2007). Each group of specimen with lice (n=20) was transferred into petridish (6 cm diameter). The test extracts were dissolved in distilled water and four concentrations were prepared arithmetically, *viz*., 12.5, 25.0, 50.0 and 100.0 mg/ml serial dilution. The petridish, containing each group of lice was charged with test extracts of various solvents. The control group was maintained with each set of experiment where the parasite was treated in a solution which contains distilled water. This petridish was kept at 25 ± 2^{o}C and 80 ± 5% Rh in the desiccators and transferred into incubator as per the guidelines of proceedings of 9th International conference on controlled atmosphere and fumigation in stored products. The average mortality % of lice in all groups was recorded.

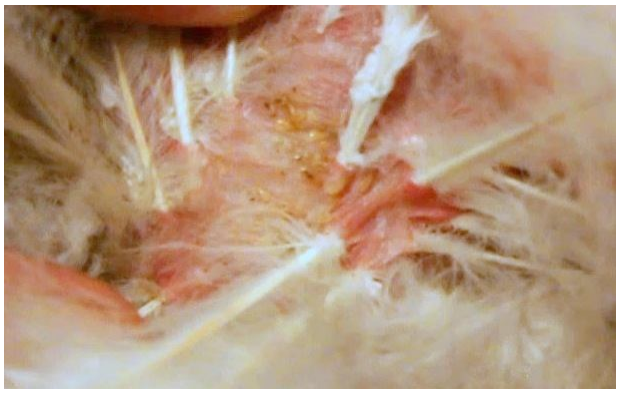

Figure 1: Lice infestation in poultry

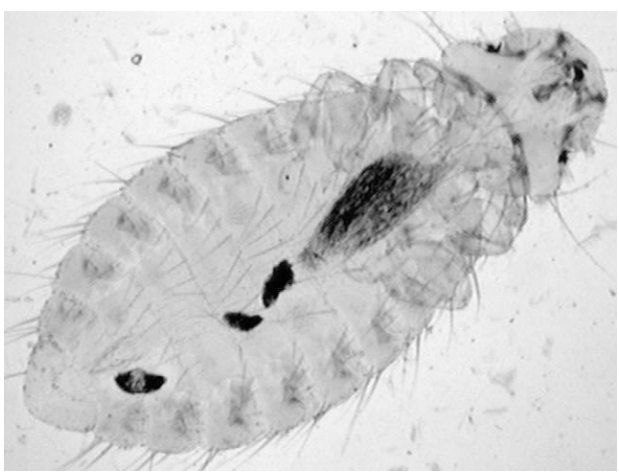

Figure 2: Menopan gallinae (4x)

2.5 *In vivo* antipediculicidal activity

In vivo antipediculicidal activity was conducted in naturally infested backyard desi poultry flock, using only ethyl acetate seed extract

of *A. muricata* Linn. after performing the *in vitro* trials. The extract from these plants was used @ 100, 50, 25 and 12.5 mg/ml concentrations. The extracts were dissolved in distilled water with 2 drops of tween-20. Four concentrations were sprayed on selected infested area with the help of sprayer. The study was conducted during hot humid season, when the burden of parasites was very high in bird premises.

3. Results

3.1 Phytochemical screening of the extract

Phytochemical analysis revealed the presence of bioactive compounds, especially tannins, saponins, flavonoids, betacyanins, quinones, cardiac glycosides, terpenoids, phenols, coumarins, steroids and alkaloids in all extracts. Ethyl acetate extract showed more positivity for tannins, saponins, flavonoids, betacyanins, phenolics and alkaloids than petroleum ether extract which showed strong positivity for tannins and alkaloids. However, methanolic extract showed no strong positivity of bioactive compounds. All the three extracts showed negativity for anthocyanin and glycosides (Table 1).

Table 1: Phytochemical analysis of seeds of *A. muricata* Linn.

Test	Solvent Inference		
	Petroleum ether	Ethyl acetate	Methanol
Tannin	+++[a]	+++[a]	+[b]
Saponin	+[b]	+++[a]	+[b]
Flavonoids	+[b]	+++[a]	-[c]
Anthocyanin	-[c]	-[c]	-[c]
Betacyanin	+[b]	+++[a]	+[b]
Quinones	+[b]	+[b]	+[b]
Glycosides	-[c]	-[c]	-[c]
Cardioglycosides	+[b]	+[b]	-[c]
Terpenoids	+[b]	+[b]	-[c]
Phenols	+[b]	+++[a]	+[b]
Coumarins	+[b]	+[b]	+[b]
Steroids	+[b]	+[b]	-[c]
Alkaloids	+++[a]	+++[a]	+[b]

a = Strong positive b = Positive c = Negative

3.2 *In vitro* antipediculicidal activity

In vitro study showed ethyl acetate had highest mortality in terms of Mean ± SE (18 ± 1.41) at 100 mg/ml, (15 ± 1.47) at 50 mg/ml, (12 ± 1.29) at 25 mg/ml and (8 ± 1.08) at 12.5 mg/ml, respectively. Highest concentration of drugs in all extract performed better than lowest concentration (Table 2).

Table 2: *In vitro* antipediculicidal activity of seed extracts of *A. muricata* Linn.

Test	Sample concentration (mg/ml)	Average mortality %
Distilled water	-	-
Petroleum ether	12.5	40
	25.0	50
	50.0	65
	100.0	80
Ethyl acetate	12.5	40
	25.0	60
	50.0	75
	100.0	90
Methanol	12.5	25
	25.0	35
	50.0	55
	100.0	60

3.3 *In vivo* antipediculicidal activity

In vivo study for antipediculicidal activity in naturally infested backyard poultry flock (Figure 3), using ethyl acetate, had good drug response at higher concentration and the flock recovered dramatically with complete wound healing.

Figure 3: *Menacanthus stramineus* (4x)

4. Discussion

4.1 Phytochemical screening

Phytochemical screening of *A. muricata* Linn. seed performed well to express the chemical constituents in ethyl acetate extracts when compared to methanolic and petroleum ether. In fact, taking into account, the activity of crude extracts and the lack of new, safe and effective acaricidal drugs in lice infestations, it is necessary to identify the acaricidal compounds of these plants (Bories *et al.*, 1991). Phytochemical screening of various plants has been reported by many workers (Raman, 2006; Gajalakshmi *et al.*, 2012). These

studies have revealed the presence of numerous chemicals including alkaloids, flavonoids, steroids, phenols, glycosides and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. Phytochemical studies showed many active compounds which are having many pharmacological activities such as anti-inflammation, antitumour activities (Pardhasaradhi *et al.,* 2004). Though the study performed, the qualitative significant of *A.muricata* Linn., the study need extraction and separation of major compounds from crude extracts as suggested in the study of (Junya *et al.,* 2006). It could be ideal to isolate the secondary metabolite efficient in drug action potential, a complete isolation procedure for active principle is mandatory for detailed investigation for antipediculicidal property.

4.2 Antipediculicidal activity

Acaricidal infestations of human beings and animals have been acknowledged to have adverse effects on the health standards with a consequent lowering of resistance to other disease. Choudhary and Vasanthi (2004) conducted *in vitro* study with *Icotina tobaccum* against *Rhipicephalus haemaphysaloides.* Further, Vatsya and Das (2004) reported *in vitro* tickicidal action of few herbal plants against *Boophilus microplus*. In this study, lice infestation in poultry causing severe drop in egg production and its successful treatment after *in vitro* and *in vivo* clinical trials are discussed. Now-a-days resistance to the available synthetic drugs is a major problem. Therefore, in recent years, a search for plant derived drugs is the primary choice of researchers, as they are believed to have less side effects and more compatible with the physiological flora (Sen *et al.,* 2010). The ethyl acetate extract of *A. muricata* Linn. seed showed complete recovery of lice infestations in poultry and premises, as supported from previous reports (Gritsanapan *et al.,* 1996; Tiangda *et al.,* 2000; Kosalge and Fursule, 2009) against anti-head lice effect in human beings.

The acaricidal activity could be detected with various natural herbal drugs, allopathic lice repellant like synthetic pyrethroids as a poultry model to assess the transfer inhibition effect of head lice (*Pediculus humanus capitis*) products (Tiangda *et al.,* 2000). Ketzis *et al.* (2014) performed comparative *in vitro* antipediculicidal efficacy of treatment in a resistant head lice population in United States. Terri *et al.* (2002) studied the antihead lice effects of *Annona squamosa* seed in humans. Rahseed *et al.* (2013) stated herbal medicines have two special characteristics which distinguish them from chemical drugs; use of crude herbs and prolonged usage. The seed extract of *A.muricata* Linn. could be ideal as an antipediculide as used along with combination of polyherbals and opined to the statement of Subramoniam (2014), stated polyherbal formulations can be better than single chemical entity drugs in many medical conditions. The multivalent and multitarget actions of mixtures of phytochemicals and standardized extracts could provide therapeutic superiority compared to single compound drugs.

5. Conclusion

Ethyl acetate extract performed well than solvents and petroleum ether, and methanol for the *in vitro* and *in vivo* antipediculicide activity. However, this present study deals with the identification, antipediculicidal activity of *A. muricata* Linn. seed (peteroleum ether, ethyl acetate and methanol) extract in backyard poultry and successful recovery, it could be ideal to use combination of polyherbals to overcome acaricide resistance. The flock premises had been insisted to change the soil and bedding materials to avoid relapse of reinfestations and effective treatment.

Conflict of interest

We declare that we have no conflict of interest.

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